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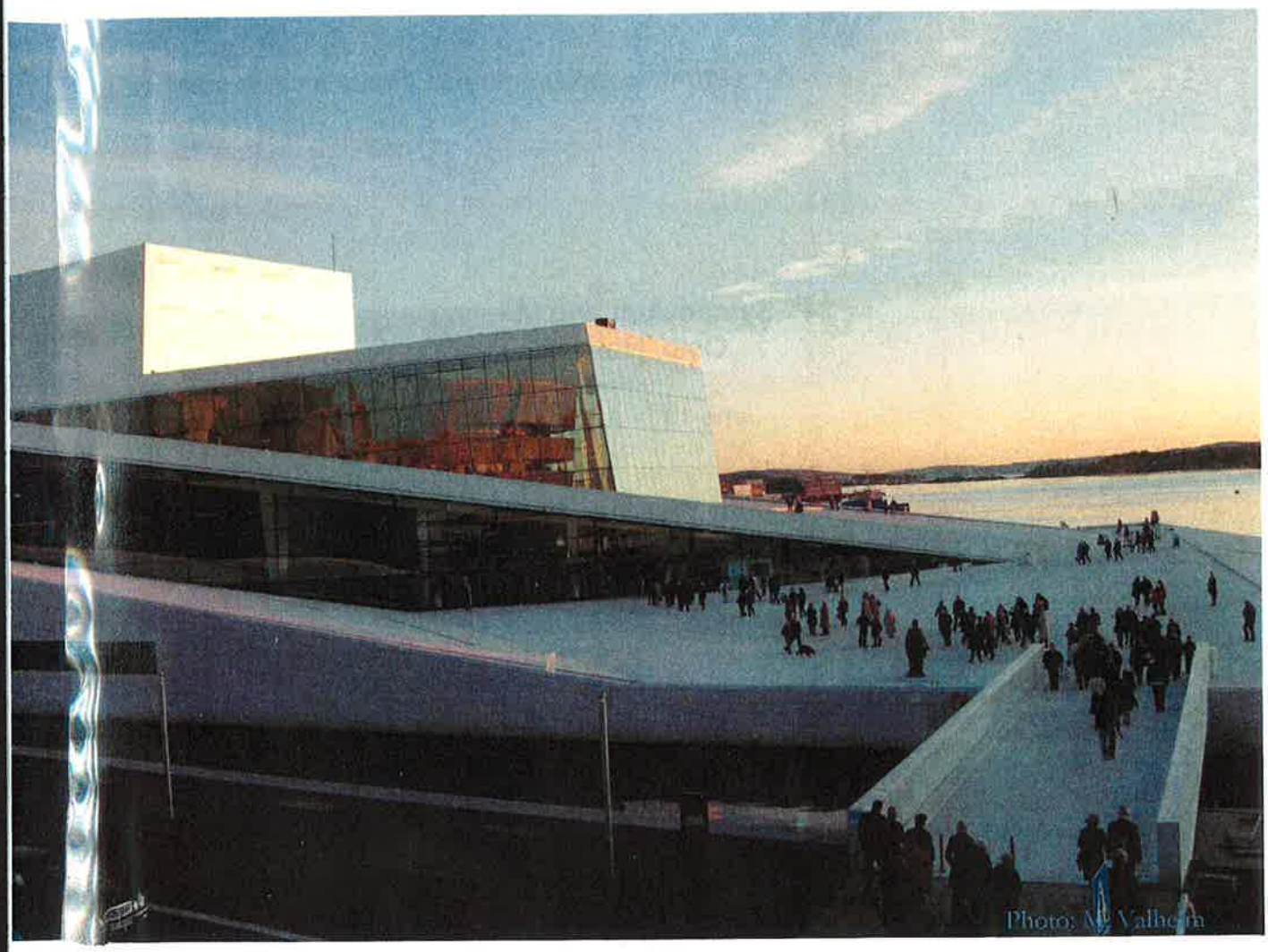

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Diarrhoea in fattening pigs

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Introduction

Intestinal infections are common diseases among growing/finishing pigs throughout the world causing substantial economic losses and decreased animal welfare. Since 1995 intestinal pathogens has been studied intensively at the National Veterinary Institute, Technical University of Denmark, with special regards to diagnosis, pathogenesis and epidemiology of *Lawsonia intracellularis,* and *Brachyspira* spp. Recently Porcine Circo Virus type 2 (PCV2) and *Fusobacterium necrophorum* have been shown as noteworthy/emergent differential diagnoses in cases of necrotizing enterocolitis. This presentation provides a short review of the main activities carried out until now with special reference to in situ diagnosis and pathology.

Methods for in situ demonstration of intestinal pathogens

Specific diagnostic methods for in situ detection of *L. intracellularis* in tissue includes immunohistochemistry (IHC) using a monoclonal antibody, Law1-DK, and application of oligonucleotide probes targeting 16S ribosomal RNA of the bacterium for fluorescent *in situ* hybridisation (FISH) (1, 2). Due to strong cross-reactions between the different species of *Brachyspira,* development of IHC methods for specific detection is difficult. Thus, for a comprehensive differentiation of porcine *Brachyspira* spp. we have developed *in situ* hybridisation methods for the specific detection of genus *Brachyspira*, *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *B. innocens* and *B. murdochii* (3, 5). In contrast to immunological methods that rely on the expression of specific markers that may not be constant, phenotypic variation does not pose a problem when rRNA is used as a target. The sensitivity of FISH is correlated to the amount of rRNA in the target organisms thus; it is strongly influenced by the physiological history and current physiological state of the bacteria e.g. starvation can result in complete lack of detectable hybridisation. Concerning *Brachyspira* spp and the use of FISH as a routine diagnostic test we recommend the colon tissue samples to be fixed in formalin as soon as possible (within half an hour) after the pig has been sacrificed. By this procedure we are able to get a high hybridisation signal as well as a well-preserved intestinal morphology for studying of the spatial distribution of the spirochetes. The effects of post-mortem autolysis of the tissue samples on the results of FISH in comparison to IHC have only been studied for *L. intracellularis*: IHC was much less susceptible than FISH to the effects of autolysis. Thus, three of nine ileum samples were FISH-negative after being kept at 20°C for 4 days, and seven were FISH-negative after 2 weeks; after 4 weeks at this temperature, however, six of the nine samples were still IHC-positive. After being kept at 4°C for 12 weeks, the majority of samples (>66%) were positive by both methods (4).

Differential diagnoses of enteritis in slaughter pigs

The applicability of *in situ* detection methods (FISH and IHC) for the demonstration of intestinal pathogens (*L. intracellularis, Brachyspira* spp, and PCV2) was investigated in formalin-fixed, paraffin-embedded tissue

samples of the intestines (5). The pigs were submitted for routine laboratory examination with suspicion of spirochaete-associated diarrhoea/colitis. All together, 113 out of 140 pigs were positive for at least one agent, 28 pigs revealed double infections and two pigs were concomitant infected by 3 pathogens (*L. intracellularis, B. hyodysenteriae* and *B. pilosicoli*). The most prevalent pathogen was *L. intracellularis* (49 positive). PCV2 associated enterocolitis was detected in 23 pigs – all histopathologically characterized by varying histiocytic infiltration (macrophages and mononuclear cells) and no proliferation of immature enterocytes as seen in proliferative enteropathy caused by *L. intracellularis*. The number of pigs positive for *B. hyodysenteriae* and *B. pilosicoli* was 37 and 13, respectively. *B. intermedia* associated colitis was demonstrated in 3 cases – all associated with catarrhal colitis including invasion of lamina propria. Colonisation of colon by *B. innocens* was demonstrated in 11 cases including 2 cases of mono-infection with catarrhal colitis and invasion of crypt and surface epithelium. *B. murdochii* was demonstrated in 6 cases including 2 cases of mono-infection with catarrhal colitis and invasion of crypt and surface epithelium. Compared to *B. hyodysenteriae* and *B. pilosicoli* infections (6, 7, 10) the histopathological changes associated with the other *Brachyspira* spp. were less severe, especially in respect to surface and crypt lesions.

The importance of *B. intermedia, B. innocens* and *B. murdochii* as intestinal pathogens has not yet been investigated thoroughly and are by many believed to be non-pathogenic. Recently, an isolate of *B. murdochii* obtained from one of the above field cases was used in an experimental study in which 8 weaned pigs were challenged reproducing catarrhal colitis in 2 animals (8). Applying FISH, *B. murdochii* organisms were found in high numbers and closely associated with the surface epithelium, only in the pigs with catarrhal colitis. The results indicate that *B. murdochii* should be regarded as low pathogenic for pigs if present in high numbers only. Similarly *B. intermedia* and *B. innocens* may be low pathogenic if present in high numbers closely associated with the colonic mucosa. However, the diagnostic importance of culturing the bacteria from feces only is uncertain, as the method is not quantitative. Thus, development of quantitative methods such as real time PCR could be useful in future studies.

The differential diagnostic importance of PCV2 associated enterocolitis was studied further in intestines from 80 pigs submitted for routine diagnostic examination with a clinical history of *L. intracellularis* associated diarrhoea (11). Histopathologically, enteritis of varying intensity was diagnosed in 64 of the pigs. Concomitant PCV2 infection was detected in 6 out of 34 (18%) intestines with *L. intracellularis* enteritis. In the 30 other cases of enteritis PCV2 infection alone was demonstrated as the etiologic diagnosis in 23 (77%). The PCV2 associated enteritis included necrotizing ileitis and colitis grossly indistinguishable from proliferative enteritis. There was no association between presence of PCV2 and other intestinal bacterial pathogens. The result demonstrates PCV2 enteritis as an important differential diagnosis to *L. intracellularis* infection in pigs aged 2 to 4 months old with clinical history of diarrhoea.

Fusobacterium necrophorum – an emerging pathogen?

The anaerobic gram-negative bacterium *Fusobacterium necrophorum* is a long slender rod. The bacterium is considered as a part of the normal flora of the digestive system occasionally causing necrotizing stomatitis and hepatitis, however, the bacterium has recently been demonstrated in outbreaks of necrotizing enterocolitis in weaned pigs grossly indistinguishable from proliferative enteritis. In the following the first case is described (9): Two pigs were submitted for routine diagnostic investigation with a history of sudden onset of diarrhea and

increased deaths within one week after weaning. At necropsy severe, acute necrotizing enterocolitis was found in both animals while the other organs appeared normal. Routine bacteriological culturing from the intestines was negative for enteropathogenic *E. coli*, *Salmonella enterica* and *Brachyspira* spp. Formalin-fixed samples of ileum and colon were immunohistochemically examined for *L. intracellularis* and PCV2 with negative result. Histopathologically, the enterocolitis was characterized by acute, deep coagulation necrosis of the mucosa with a clear demarcation zone to the vital part of the mucosa. The necrotic tissue was found severely infiltrated by long slender rods. Thus, intuitively we examined the intestinal tissue samples for *F. necrophorum* by FISH. Applying a species-specific oligonucleotide probe targeting 16S rRNA the long rods infiltrating the ileal as well as colonic mucosa of both animals were identified as *F. necrophorum*. Within the last year the bacterium has been demonstrated in another 3 herds in cases of necrotizing enterocolitis otherwise negative for intestinal pathogens. The result indicates that *F. necrophorum* in some cases should be a primary intestinal pathogen, and that it is easily demonstrated by FISH in formal-fixed tissue samples.

In conclusion, application of IHC and FISH using oligonucleotide probes targeting ribosomal RNA for detection and identification of intestinal pathogens in their natural environment is recommend, especially concerning opportunistic and low pathogenic organisms as a reliable technique for both research and routine purpose.

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